

iGEM TU/e 2015

Biomedical Engineering

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InterLab Study: Agar plates

Table of contents

Agar plates	1	Agar Plates	3
	1.1	Preparation of LB-agar	3
	1.1.1	Materials	3
	1.1.2	Setup & Protocol	3
	1.2	Pouring the plates	3
	1.2.1	Materials	3
	1.2.2	Setup & Protocol	4

1 Agar Plates

1.1 Preparation of LB-agar

Estimated bench time: 20 minutes

Estimated total time: 3 hours

Purpose: Making LB-agar for the pouring of LB-agar plates.

1.1.1 Materials

- Autoclave
- Autoclave tape
- Bacto-agar
- Balance
- Bottle (glassware)
- H₂O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

1.1.2 Setup & Protocol

With 200 ml LB-agar you can make 8 plates (1 l for ~40 plates).

- For 200 ml LB-agar, the correct amounts are:
 - 1 g yeast extract
 - 2 g NaCl
 - 2 g peptone
 - 3 g bacto-agar
- Collect them in in a bottle and add 200 ml of H₂O.
- Autoclave the LB-agar at 121 °C for 20 minutes (sterilisation).

1.2 Pouring the plates

Estimated bench time: 20 minutes

Estimated total time: 80 minutes

Purpose: Making LB-agar plates where bacteria with an additional plasmid can grow.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

1.2.1 Materials

- Antibiotic stock
- Autoclaved LB-agar
- Bunsen burner
- Petri-dishes
- Pipettes and tips

1.2.2 Setup & Protocol

- After autoclaving the LB-agar (at 121 °C for 20 minutes), let the agar cool down to ~50 °C (autoclave can be opened at 90 °C). Make sure the agar does not start solidifying.
- Add antibiotic stock (200 µl for 200 ml) to the liquid LB-agar and slowly mix.
- Pour the LB-agar in the petri-dishes until the bottom is well covered. Work near the Bunsen burner flame.
- Close the lid after filling the plate. Let the agar solidify for ~1 hour on the bench.
- Transfer the plates to a bag, in which they should be placed upside down.
- Store the plates in the fridge (4 °C).